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A STUDY OF THE ENVIRONMENTAL CONDITIONS INFLUENCING THE DEVELOPMENT OF STEM RUST IN THE ABSENCE OF AN ALTERNATE HOST

1. THE VIABILITY OF THE UREDINIOSPORES OF PUCCINIA GRAMINIS TRITICI FORM III

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A STUDY OF THE ENVIRONMENTAL CONDITIONS INFLUENCING THE DEVELOPMENT OF STEM RUST IN THE ABSENCE OF AN ALTERNATE HOST[1]

I. THE VIABILITY OF THE UREDINIOSPORES OF PUCCINIA GRAMINIS TRITICI FORM III

GEORGE L. PELTIER

INTRODUCTION

No exact data are at hand regarding the influence of temperature and relative humidity on the viability of the urediniospores of stem rust under controlled conditions. What statements do appear in the literature are general and are of no specific value in that no stated temperature or relative humidity is given. The few chapters devoted to the subject of the viability of the urediniospores by Levine[2] in his review of the literature on "The Epidemiology of Cereal Rusts" may be cited as an illustration of our lack of knowledge on this subject.

In order to determine more accurately the viability of the urediniospores of stem rust of wheat under controlled conditions, a modification of the method developed by the writer[3] in his viability study of *Pseudomonas citri* Hasse was employed. Briefly, this consisted of subjecting uredinia of approximately the same age on detached wheat seedling leaves to various combinations of constant temperatures and relative humidities. At weekly intervals, both germination tests of the urediniospores in the laboratory and infection tests in the greenhouse were made.

APPARATUS USED

Temperature controls.—For temperatures of 25° and 30° C., ordinary bacteriological incubators were employed. For tempera-

[1]Arrangements have been made whereby the publication of the various phases of this project will appear from time to time as Research Bulletins of the Station. Thus, all publications by the author dealing with this project will be issued from one source, so that uniformity in the presentation of the results will be assured.

[2]Levine, M. N. The Epidemiology of Cereal Rusts in General and of the Black Stem Rust in Particular, p. 33-34, 1919. Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture. Mimeographed.

[3] Peltier, G. L. Influence of Temperature and Humidity on the Growth of *Pseudomonas citri* and its Host Plants and on Infection and Development of the Disease. Jour. Agr. Research 20:488-491, 1920.

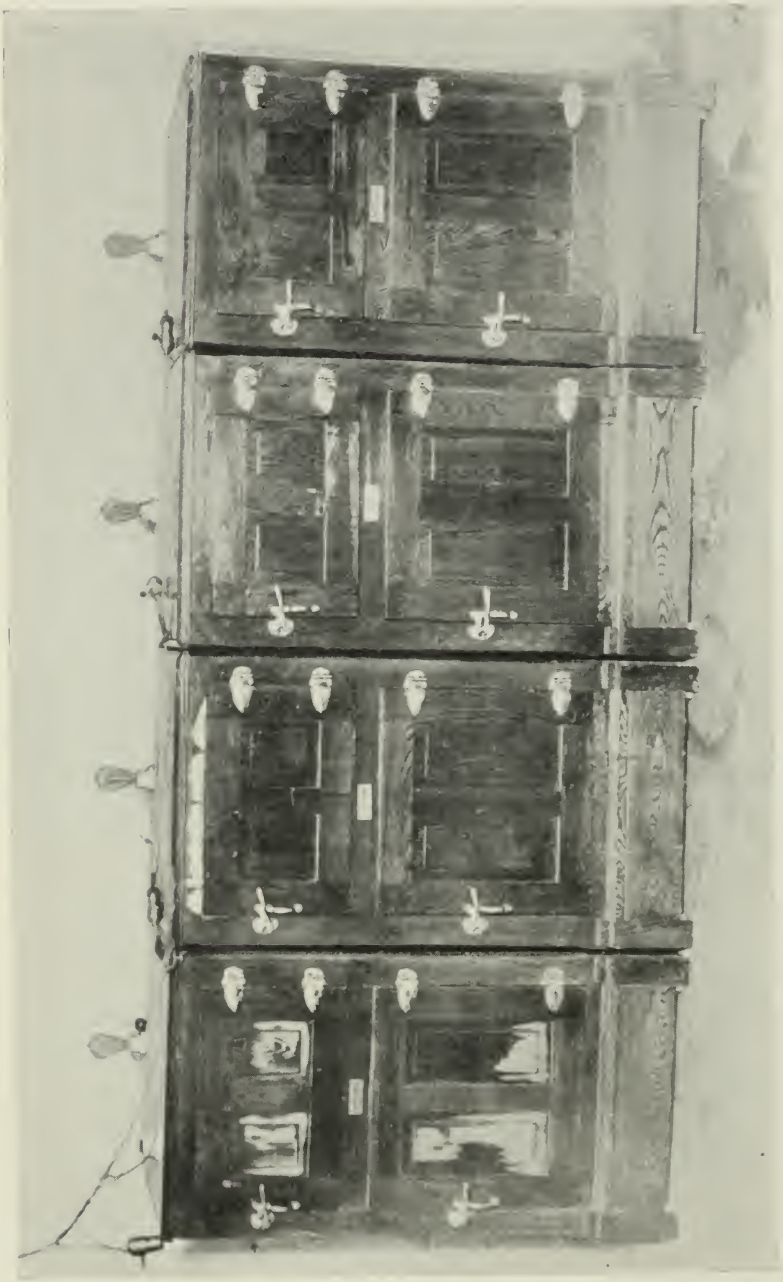

FIG. 1.—Series of incubators in cold storage room.

tures below 25° C., a highly satisfactory method was devised. Mr. Robert W. Goss, my associate, shares equally in the gradual development of this method of maintaining low temperatures. In developing this method, we went on the principle that it is much easier to maintain constant temperatures in incubators above the temperature of the surrounding medium than it is to maintain temperatures lower than that of the room in which the incubators are kept.

Four two-door ice boxes (14 by 22 by 36 inches, inside measurement) were obtained, the ice compartments dismantled, and two extra shelves put in each box so that the working capacity of the boxes was doubled (fig. 1).

These boxes were equipped with heaters which were made as follows: Chromel wire was wound on two strips of transit and fastened to the underside of a large (12 by 19 in.) transit plate having a row of holes in the center the length of the plate. The whole was then set up from the floor of the boxes (2½ in.) by means of porcelain insulators.

The chromel wire was connected up in parallel. The warm air came up thru the holes in the transit plate and also along the walls of the boxes. In this way a very uniform temperature was maintained thruout the box so that there were no measurable differences between the temperatures on the upper and lower shelves.

For constant temperature controls, the thermostats made by the Chicago Surgical and Electric Company were employed. These were fastened in the center of the back wall of the boxes. The necessary electric connections and pilot lamps completed the equipment (fig. 2).

The incubators were then installed in a cold storage room which was maintained at a more or less constant temperature of 4° C. or slightly below. The thermostats in the incubators were so set that temperatures of 5°, 10°, 15°, and 20° C. were maintained in the respective incubators. In no instance did the temperature in any one of the incubators vary more than one degree either way during the course of the experiment.

Readings were made three times a day and very little regulation of the thermostats was required, altho it was necessary to adjust and file the contact points occasionally.

During one year's experiments, even tho all the incubators were maintained at temperatures above that of the cold storage room, we could notice no effect on the constancy of the

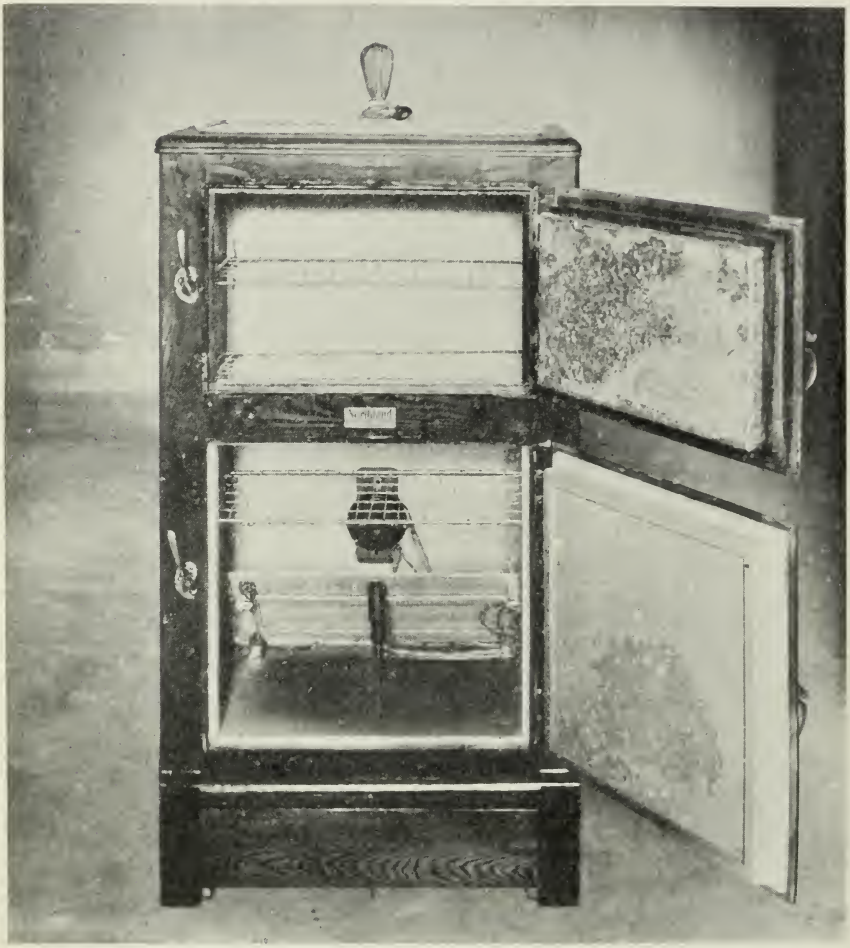

FIG. 2.—Interior of incubator showing heater, thermostat, and shelves.

temperature of this room. We feel sure that this method will solve the low temperature problem for the pathologist, at a cost which is not at all excessive ($50 per box, completely equipped), provided, of course, there is a cold storage plant available.

Humidity controls.—The method used in obtaining the desired relative humidities was essentially the same as that described by the writer[1] in his viability study of *Pseudomonas citri*

[1] *Loc. cit.*

Hasse. The method pursued in this experiment was as follows: Silk threads were stretched across an aluminum wire frame (B. & S. No. 20) 2⅛ inches square, with legs 1¾ inches high enclosed in glass stockings of the same height. These frames were placed in ordinary preparation dishes, 2 inches high and 3½ inches in diameter with loosely fitting covers resting on a shoulder; and sterilized in the autoclave. These dishes were then filled with the sulfuric acid solution up to the shoulder of the dish so that the threads were about one-fourth of an inch from the liquid. The leaves with rust pustules were placed on these threads.

These dishes were placed in larger Koch moist chambers with ground glass lids, and the solution poured into them until the liquid in both dishes was the same level. The covers of the smaller dishes were then quickly removed and the lids of the outer dishes vaselined and made air-tight (fig. 3).

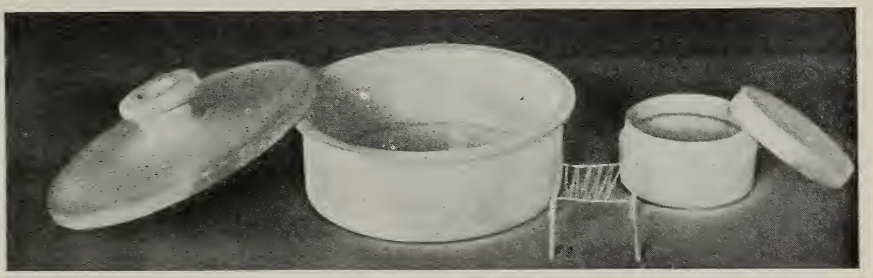

FIG. 3.—Relative humidity chambers, with accessories used in viability experiments.

For the various sulfuric acid concentrations and respective relative humidities, the table published by Stevens[1] was used. The specific gravity of all solutions was determined by a Twadell hydrometer, with the temperature of the solution at 15° C. At the conclusion of the experiment, the solutions were rechecked at the same temperature and in no case was there a variation of more than one or two points on the Twadell scale.

Incubation chambers.—Owing to the large amount of rust material needed for the experiment, and the desirability of maintaining a constant temperature during incubation, the following apparatus was used. The top of a "Wisconsin temperature tank"

[1]Stevens, Neil E. A Method for Studying the Humidity Relations of Fungi in Culture. Phytopathology 6:428-432. 1916.

was removed together with the cans, a false bottom made of slats placed in the tank about 12 inches below the top, and water brought up to within ¼ or ½ of an inch of this false bottom. By means of a heater with a thermostat connection, the water could be brought to the temperature desired. Over the top of the tank, a large pane of glass was closely fitted. Owing to the large volume of water present, these modified tanks served most admirably for incubation chambers, both from the standpoint of a uniform temperature and from that of the production of a heavy film of water on the leaves. A tank thus fitted up held in the neighborhood of 75 3½-inch pots.

PROCEDURE

On February 1, 1922, about 2,000 Little Club (C. I. No. 4066 from Moccasin, Montana, 1921) wheat seedlings, seven days old, were inoculated with two weeks old material of *Puccinia graminis tritici* Form III, a stock culture of which was kindly furnished the writer by Dr. E. C. Stakman of the Minnesota Agricultural Experiment Station in the fall of 1921. During the season it was transferred each week to Little Club wheat. It may be stated further that it was the only strain of rust introduced into the greenhouse during the season of 1921-1922.

As usual in rust inoculation of wheat seedlings, the plants were trimmed before inoculation and five such trimmed plants were retained in each pot. The plants were sprayed with water by means of an atomizer before inoculation and the wet fingers drawn over leaves just prior to inoculation.

A composite inoculum was obtained by shaking the spores from the pustules on to a glass plate. While the waste of rust material is rather large by this method, it is counterbalanced by the more uniform and higher percentage of successful infections obtained. As Form III was the only rust material present in the greenhouse, no necessity for the sterlization of the flat needle after each inoculation existed. Coupled with the fact that it was not necessary to stop and obtain spores from the pustules on the leaves with the needle at each operation, this greatly facilitated the work. Thus, with an assistant to get the plants ready and place the inoculated plants in the incubation chambers, the writer could inoculate from 2,000 to 2,500 plants in six to eight hours.

The inoculated plants were incubated for 48 hours in the incubation chambers, which were maintained at a constant tem-

perature of 25° C. At the end of this period the plants were taken out, trimmed, and benched in a greenhouse equipped with a Johnson Service Company temperature control. During the period of 12 days that the plants were benched, the daily mean temperature of the house was 24° C. The plants were trimmed every third day.

Flecking was noted on some of the plants February 5, and by February 7 rust pustules were beginning to appear. On February 14, 13 days after inoculation, the infected leaves were cut off and approximately 30 leaves were placed on the silk threads in each humidity chamber already described.

At the end of each week, one or two leaves, depending on the number of uredinia present per leaf, were withdrawn from each dish. Duplicate germination tests were made. The remainder of the rust material was used for the weekly infection tests in the greenhouse.

A composite sample of the spores from each leaf was germinated in a drop of distilled water placed on a cover glass and inverted over a glass ring, mounted on a glass slide. These were placed in a large, moist chamber and incubated for 24 hours at room temperature. The percentage of germination was determined by counting a total of 200 spores at various points in the microscopic field.

In the greenhouse, five seedling plants were inoculated with the remainder of the rust material from each dish. About 10 or 12 days after inoculation the number of pustules per leaf was recorded.

EXPERIMENTAL DATA

Constant temperatures of 5°, 10°, 15°, 20°, 25°, and 30° C. were used. A set of 11 moist chambers was placed at each temperature, the air in each chamber being maintained at a constant relative humidity by means of sulfuric acid solutions. Thus, at each temperature, there was a series of relative humidities ranging from 0 to 100 per cent, at approximately 10 per cent intervals. The experiment was run over a period of 16 weeks, at the end of which the rust material was exhausted.

Germination tests.—The results of the germination tests are given in Table 1. As none of the urediniospores held at 30° C. germinated, the results are not shown in the table.

No germination of the urediniospores occurred at either the high or the low relative humidities at 25° C. At the medium relative humidities some germination took place, but the percent-

TABLE 1.—*Viability of the urediniospores of Puccinia graminis tritici Form III.*

Approximate relative humidity	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks	After 6 weeks	After 7 weeks	After 8 weeks	After 9 weeks	After 10 weeks	After 11 weeks	After 12 weeks	After 13 weeks	After 14 weeks	After 15 weeks	After 16 weeks
Per cent								*25° C.*								
100.0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.9		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80.5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70.4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60.7		+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49.0		+	+	—	—	0	0	0	0	0	0	0	0	0	0	0
38.0		0	±	—	0	0	0	0	0	0	0	0	0	0	0	0
29.5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10.5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Per cent								*20° C.*								
100.0	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.9	±	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
80.5	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70.4	++	0	+	0	—	0	0	0	0	0	0	0	0	0	0	0
60.7	++	+	++	++	+	±	—	—	—	0	0	0	0	0	0	0
49.0	++	+	++	++	++	+	+	±	—	—	—	—	0	0	0	0
38.0	++	+	+	+	+	—	—	—	—	0	0	0	0	0	0	0
29.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.5	++	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
10.5	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	++	+	+	0	0	±	—	—	0	0	0	0	0	0	0	0
Per cent								*15° C.*								
100.0	±	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.9	—	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0
80.5	++	0	—	—	—	0	0	0	0	0	0	0	0	0	0	0
70.4	—	0	—	—	—	±	0	0	0	0	0	0	0	0	0	0
60.7	++	+	++	++	0	+	++	+	+	—	±	—	—	±	—	0
49.0	++	+	++	++	++	++	++	++	++	+	—	—	—	0	+	—
38.0	++	+	++	++	+	+	++	++	0	+	—	—	—	0	—	—
29.5	++	+	—	—	0	0	0	0	0	0	0	0	0	0	0	0
21.5	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
10.5	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	++	—	+	+	+	+	±	—	0	0	0	0	0	0	0	0
Per cent								*10° C.*								
100.0	±	—	0	—	0	0	0	0	0	0	0	0	0	0	0	0
89.9	±	—	0	±	0	+	±	—	0	—	—	0	0	0	0	0
80.5		+	++	++	++	+	+	—	—	±	—	0	0	0	0	0
70.4	++	++	+	+	++	+	+	+	+	+	±	—	0	±	0	—
60.7	+	—	+	+	+	++	++	++	++	++	+	+	+	++	—	—
49.0	++	+	++	++	++	+	++	++	++	++	++	—	+	+	+	+
38.0	+	+	++	—	++	++	++	++	—	±	+	+	—	++	—	±
29.5	+	+	++	+	0	—	—	—	0	—	0	—	0	0	0	0
21.5	+	±	++	±	0	—	0	0	0	±	0	0	0	0	0	0
10.5	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	+	—	+	+	+	+	±	0	—	—	—	0	0	0	0	0
Per cent								*5° C.*								
100.0	—	—	0	±	0	0	0	0	0	0	0	0	0	0	0	0
89.9	—	—	—	0	0	±	0	0	0	0	0	0	0	0	0	0
80.5	++	±	++	++	++	—	++	+	+	—	±	0	—	0	0	0
70.4	++	++	++	++	++	++	++	++	+	++	++	+	+	++	++	—
60.7	++	++	++	++	++	++	++	++	++	++	++	—	++	+	++	+
49.0	+	±	++	—	+	±	++	++	—	++	—	—	+	++	++	—
38.0	++	+	++	++	++	++	++	+	+	++	++	±	+	+	±	±
29.5	++	+	+	—	+	+	±	+	—	0	—	0	0	0	0	0
21.5	+	++	+	+	+	±	—	—	—	—	0	0	0	0	0	0
10.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	—	—	0	—	0	0	0	0	0	0	0	0	0	0	0	0

0 = No germination.
— = 1 to 5 per cent germination.
± = 5 to 10 per cent germination.
\+ = 10 to 40 per cent germination.
++ = above 50 per cent germination.

age of germination decreased rapidly with an increase of the time interval. At this temperature the maximum length of time that the spores were viable, five weeks, occurred at the relative humidity of 49.0 per cent.

At 20° C., the urediniospores were viable for a greater range of relative humidities and over a longer period than at 25° C. At approximately 100 per cent relative humidity, the spores were viable for one week. A higher percentage of germination over a longer period of time was obtained with each drop in humidity until 49.0 per cent was reached, where the spores were viable at the end of 11 weeks. Again, with each succeeding drop in the relative humidity there was a general decrease in the percentage of spores germinating and they remained viable for a shorter length of time. As at 25° C., the spores gave the highest percentage of germination and were viable longer at the medium humidities.

At 15° C., the urediniospores were viable over longer periods at all humidities than at 20° C. However, there was the same relation between the viability of the spores at the various relative humidities as at 20° C. At the relative humidities of 49.0 to 60.7 per cent the spores germinated at the end of 10 weeks.

At 10° C., the spores gave a higher percentage of germination and were viable for more extended periods at nearly all humidities than at 15° C. At the end of the sixteenth week at the relative humidities of 38.0, 49.0, and 70.4 per cent, a fairly high percentage of spores were still germinating.

At 5° C., the viability of the spores at the medium humidities was still fairly high at the conclusion of the experiment. At the relative humidities above 70.4 per cent, the spores were viable for about as long as the corresponding relative humidities held at 10° C. On the other hand, at relative humidities below 38.0 per cent the spores were viable for a shorter time than at 10° C. These results show that we have reached a point where, at the lower relative humidities held at 5° C., the spores are not as viable as long as at the corresponding relative humidities at 10° C.

If the rust material had been available for further tests, the spores submitted to the medium humidities and temperatures of 5°, 10°, and 15° C. would doubtless have remained viable for a considerable time, as the percentage of germination was still high at the conclusion of the experiment.

It will be noted from the table that in the majority of instances there is a gradual decrease in the percentage of germina-

tion with an increase in the time interval and that the percentage of germination remains small over a period of several weeks.

Rather peculiar results were obtained at the relative humidity of 10.5 per cent at all temperatures below 25° C. For some unknown reason, a lower percentage of germination was obtained at this point than at the relative humidities of 0 and 21.5 per cent. For example, at 10.5 per cent relative humidity, no spores were found capable of germinating after two weeks at any temperature, while the number of spores germinating at this relative humidity was low in all cases. On the other hand, at the relative humidities of 0 and 21.5 per cent a larger number of spores germinated over a longer period of time, the percentage of germination and the time interval depending somewhat on the temperature at which they were held.

The results of the germination tests show that we have a very definite relation between the viability of urediniospores and the environmental factors of the temperature and relative humidity. No doubt, with the use of a larger number of germination tests and more uniformity of rust material, the results could be expressed in mathematical ratios.

Infection tests.—The purpose of checking up the germination tests with infection tests was twofold. In the first place, it served as a check on the methods used and the results obtained with the germination tests. Further, it afforded an opportunity to determine whether urediniospores still able to germinate were capable of entering the host and producing a pustule.

The results of the infection tests showing the length of time, after being submitted to various temperatures and relative humidities, that the spores were capable of producing infection are given in Table 2.

As a rule the results of the infection tests are in accord with those obtained in the germination tests. In most instances where 10 per cent or more of the spores were viable in the germination tests, infection was successful. There is also a definite relation between the percentage of germination and the number of pustules per leaf and number of leaves infected.

Few or no infections were obtained with any inoculum which showed less than 10 per cent germination. Whether this was due to the small number of viable spores in the inoculum, or whether the spores upon germination were unable to produce infection, is not clear. However, it was observed that as the

TABLE 2.—*Length of time after being exposed to the various temperatures and relative humidities that the urediniospores of Puccinia graminis tritici Form III were capable of producing infection.*

Approximate relative humidity	25° C.	20° C.	15° C.	10° C.	5° C.
Per cent	*Weeks*	*Weeks*	*Weeks*	*Weeks*	*Weeks*
100.0	0	1	0	1	0
89.9	0	0	0	0	1
80.5	0	1	1	2	2
70.4	0	1	2	16	16
60.7	2	0	14	14	16
49.0	2	1	15	16	16
38.0	0	1	14	14	15
29.5	0	1	1	3	4
21.5	0	0	1	2	4
10.5	0	0	1	0	0
0.0	0	1	1	5	0

percentage of germination decreased from week to week, the germ tubes became shorter, narrower, and the protoplasm was not as dense as in normal germ tubes. Further, when such inoculum was used, hypersensitive flecks were produced on the leaves, indicating that, while the spores were capable of germinating and sending a germ tube into the tissues, the resistance offered by the host cells was greater than the fungus could overcome. Judging from the data obtained, we can assume that a point is reached where the urediniospores are so attenuated under certain environmental conditions that while they are still able to germinate they are unable to produce infection.

DISCUSSION

The problem of the viability of the urediniospores of stem rust under field conditions is a question of their ability to overwinter in the North or oversummer in the South. The consensus of opinion is that the urediniospores of *Puccinia graminis tritici* do not overwinter in the North. This is borne out by the results obtained by the writer during the past two winters in field experiments conducted at Lincoln. Rust epidemics were started in the fall and urediniospores were found that germinated readily as far into the winter as January, but after this time it was exceedingly

difficult to find urediniospores, and what few were found did not germinate.

Infections in the late fall on winter wheat are usually confined to the lower and outer leaves and these are the first leaves which dry up under adverse environmental conditions. Thus, the use of detached leaves in the experiment reported was the nearest approach possible, under artificial conditions, to what actually occurs in the field. The results, while they do not touch the whole of the overwintering question, do show that down to certain temperatures the urediniospores are viable for long periods of time at the prevailing relative humidities encountered in the field in the fall in the winter wheat belt. Experiments will be carried out this coming year with temperatures below freezing. The effect of alternate freezing and thawing on the viability of urediniospores will also be determined.

The results show clearly that at constant temperatures below the mean temperatures prevailing during the summer months in the South the urediniospores are not viable for any great length of time at any relative humidity. At first thought one would say that the results show that oversummering could not take place in the South. Further, the mean temperatures prevalent in the South during the summer months are near the maximum at which germination of the urediniospores can take place. Where, then, do the urediniospores that produce infection in the fall on winter wheat in the South come from?

The mortality of the free urediniospores must be extremely high at all times. The rate of mortality would naturally vary with the environmental factors encountered. Further, a definite set of conditions must be at hand for infection to take place. These conditions are not always present, so that the death rate of the viable spores would further be increased. Thus, it is safe to assume that only a small proportion of the countless number of urediniospores produced actually infect the host, except during the short periods when the conditions for successful infection are at hand.

It should be distinctly understood that the generalizations offered above are based on the results obtained with only one biologic form. Will other biologic forms of stem rust react in the same way, or will they behave differently to stated temperatures and relative humidities by having a lower or higher percentage of germination and a shorter or longer period of viability? To determine these points, it is the purpose of the writer to continue this work with other biologic forms of stem rust.

CONCLUSIONS

1. The influence of relative humidity on the viability of the urediniospores of *Puccinia graminis tritici* Form III is very pronounced and closely associated with temperature.

2. At high relative humidities, depending somewhat on the temperature, the percentage of germination is low and the period over which the spores are viable is very short.

3. The same relation exists at the low relative humidities except that it is not so pronounced and is more variable.

4. The highest percentage of germination and the longest viable period of the spores occur at the medium relative humidities. High temperatures lower the percentage of germination and shorten the viable period while at low temperatures the opposite occurs, at the medium relative humidities.

(3M)